sequences contain restriction sites (Bsa I and Eco47 III, BamH I and EcoR I) for subsequent cloning, expression, and insert removal. The PCR protocol was as follows: first cycle, 95° C, 3 min; 52° C 1 min; 72° C 1 min; 4 cycles, 94° C, 45 sec; 52° C, 45 sec; 72◦ C, 1 min; 30 cycles, 94° C, 45 sec; 55° C, 1 min; 72° C, 1 min.

Please delete the paragraph on page 23, lines 30-31, thru page 24, lines 1-7, and replace it with the following paragraph:

Polyclonal Antibody Production. Antibovine leptin antibodies will be necessary for a thorough evaluation of the role of leptin in growth and reproduction, and may prove to be useful for manipulation of the physiological activities of leptin *in vitro* and *in vivo*. Two polyclonal antibodies were prepared. Initially, a primary antibody was developed using a synthetic 13-amino acid polypeptide (VPIQKVQDDTKTL) (residues 1-13 of SEQ ID NO: 4) corresponding to the N-terminal of bovine leptin. The polypeptide was conjugated to keyhole limpet hemocyanin (KLH) prior to immunization of the rabbits. A second polyclonal antibody was prepared using recombinant bovine leptin prepared as described above. The recombinant protein was gel-purified and the appropriate band excised and used for antibody production.

REMARKS

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date February 13, 2003

By Alan E. Wagner
Alan E. Wagner
Registration No. 45188

WHYTE HIRSCHBOECK DUDEK S.C.
111 East Wisconsin Avenue, Suite 2100
Milwaukee, Wisconsin 53202
Customer No. 022202

MARKED UP VERSION ATTACHED TO AMENDMENT IN SERIAL NO. 09/928,522

Marked up version of page 4, lines 15-30, is below:

FIG. 1 depicts the bovine leptin cDNA nucleotide sequence (top) (SEQ ID NO:3) and predicted amino acid sequence **(SEQ ID NO: 4)** (bottom) for the coding region minus the secretory signal.

FIG. 2A shows a comparison of the bovine leptin cDNA nucleotide sequence (SEQ ID NO:3) with the human nucleotide sequence **(SEQ ID NO: 5)**.

FIG. 2B shows a comparison of the bovine leptin cDNA nucleotide sequence (SEQ ID NO:3) with the murine nucleotide sequence**s** **(SEQ ID NOS 6 & 9)**.

FIG. 3A shows a comparison of the predicted bovine leptin amino acid sequence (SEQ ID NO:4) with the human leptin amino acid sequence **(SEQ ID NO: 7)**.

FIG. 3B shows a comparison of the predicted bovine leptin amino acid sequence (SEQ ID NO:4) with the murine leptin amino acid sequence **(SEQ ID NO: 8)**.

FIG. 4 depicts a portion of the actual bovine leptin amino acid sequence **(residues 1-30 of** SEQ ID NO:4) which is an N-terminal sequence comprising 30 amino acids.

FIG. 5A shows a comparison of the actual N-terminal bovine leptin amino acid sequence (**residues 1-30 of** SEQ ID NO: [~~7~~] **4**) with the human leptin amino acid sequence **(residues 22-51 of SEQ ID NO: 7)**.

FIG. 5B shows a comparison of the actual bovine leptin amino acid sequence (**residues 1-30 of** SEQ ID NO: [~~8~~] **4**) with the murine leptin amino acid sequence **(residues 22-51 of SEQ ID NO: 8)**.

Marked up version of paragraph on page 16, lin s 18-31, thru page 17, lines 1-4, is below:

2. PCR and Primer Information:

The single-stranded bovine cDNA pool was used as a template to amplify bovine leptin cDNA in a PCR reaction with synthetic DNA primers based on the published mouse leptin cDNA sequence. Two pairs of oligonucleotide degenerate primers specific for the human and murine leptin gene were designed and synthesized (DNA International, Lake Oswego, OR). The primers were designed to amplify the coding region of the bovine leptin gene, excluding the secretory signal at the 5'-terminal of the coding region). The forward primer has a sequence of 5'-GGA TCC GGT CTC AGG CCG TGC CYA TCC ARA AAG TCC-3' **(SEQ ID NO: 1)** (contains a BsaI site), and the reverse primer has a sequence of 5'-GAA TTC AGC GCT GCA YYC AGG GCT RAS RTC-3' **(SEQ ID NO: 2)** (contains a Eco47III site), where R=(A,G), S=(C,G), Y=(C,T). PCR was performed using the following conditions: 1X PCR buffer, 1.5 mM $MgCl_2$, 1 iM primers, 0.2 mM dNTPs and 5 units of Taq polymerase per 100-il reaction. A total of 32 cycles were run with following cycling conditions: 94C, 1 min; 55C, 1.5 min; and 72C, 1.5 min. After running the PCR product on a 1% agarose gel, a band of 449 base pairs was obtained from the PCR-amplified bovine single-stranded cDNA as depicted in Figure 6. Specifically, lane 1 of Figure 6 contains the 449 base pair bovine leptin cDNA, lane 2 contains the pASK75 vector DNA, and lane 3 contains standard 100 base pair ladder. The size of the PCR product was consistent with the predicted size of the coding region of the bovine leptin gene. This PCR product was verified in a secondary PCR procedure.

Marked up version of paragraph on page 18, lines 6-22, is below:

C. DNA and Protein Sequencing

Sequencing of the insert DNA (both sense and antisense strands) was performed by a commercial laboratory (National Bioscience, Inc.) using the standard Sanger's dideoxy-nucleotide method. Briefly, the PCR product containing the 449 bp band was separated on a 1% low-melting-agarose gel. The 449 bp band was cut from the gel, further purified using a Genecleaning kit (Bio101, Inc, Vista, CA), and submitted for sequencing. The sequences were then compared with the Genebank and other databases using the GCG software. The sequence data confirm that the 449 bp product from two independent clones shares approximately 87.6% homology with the human leptin cDNA (FIG. 2A, SEQ ID NO: ~~[3]~~ **5**) and 84.9% with the mouse leptin cDNA (FIG. 2B, SEQ ID NO: ~~[3]~~ **6**). The predicted amino acid sequence also shares approximately 87% homology with the human leptin protein (FIG. 3A, SEQ ID NO: [4] **7**) and approximately 86.3% homology with the murine leptin protein (FIG. 3B, SEQ ID NO: [4] **8**). Moreover, a portion of the predicted amino acid sequence was confirmed through amino terminal sequencing. Specifically, 30 amino acids comprising the N-terminal sequence have been obtained (FIG. 4, **residues 1-30 of** SEQ ID NO:4). The actual amino acid sequence (i.e., the N-terminal sequence (FIG. 4, **residues 1-30 of** SEQ ID NO:4)) shares approximately 100% homology with the human leptin protein (FIG. 5A, **residues 22-51 of** SEQ ID NO:7), and approximately 100% homology with the murine leptin protein (FIG. 5B, **residues 22-51 of** SEQ ID NO:8).

Marked up version of paragraph on page 21, lines 27-31, thru page 22, lines 1-10, is below:

Reverse Transcription and Polym rase Chain R action Amplification. Subcutaneous adipose tissue was obtained by surgical biopsy from the tail-head depot of a gestating cow using local anesthesia. Total RNA was extracted using a modified method based on Chomczynski and Sacchi's acidic guanidine thiocyanate extraction (Chirgwin, J.J., A.E. Przbyla, R.J. MacDonald, and W.J. Rutter, 1979, Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease, Biochemistry 18:5294-5299). Poly A^+ RNA was purified using oligo (dT) cellulose mini-columns purchased commercially. For the reverse transcription reaction, an oligo $d(T)_{12-18}$ primer and 2 ìg poly A^+ RNA were used. In the subsequent polymerase chain reaction (PCR) amplification, the following primer sequences were used: 5' -GAA TCC GGT CTC AGA CCG TGC C[Ʉ]YA TCC ARA AAG TCC-3' **(SEQ ID NO: 1)** (sense) and 5' -GAA TTC AGC GCT GCA YYC AGG GCT RAS RTC-3' **(SEQ ID NO: 2)** (antisense), where R=A, G; Y=C, T; S=C, G. These primer sequences contain restriction sites (Bsa I and Eco47 III, BamH I and EcoR I) for subsequent cloning, expression, and insert removal. The PCR protocol was as follows: first cycle, 95° C, 3 min; 52° C 1 min; 72° C 1 min; 4 cycles, 94° C, 45 sec; 52° C, 45 sec; 72◦ C, 1 min; 30 cycles, 94° C, 45 sec; 55° C, 1 min; 72° C, 1 min.

Marked up version of paragraph on page 23, lines 30-31, thru page 24, lines 1-7, is below:

Polyclonal Antibody Production. Antibovine leptin antibodies will be necessary for a thorough evaluation of the role of leptin in growth and reproduction, and may prove to be useful for manipulation of the physiological activities of leptin *in vitro* and *in vivo*. Two polyclonal antibodies were prepared. Initially, a primary antibody was developed using a synthetic 13-amino acid polypeptide (VPIQKVQDDTKTL) **(residues 1-13 of SEQ ID NO: 4)** corresponding to the N-terminal of bovine leptin. The polypeptide was conjugated to keyhole limpet hemocyanin (KLH) prior to immunization of the rabbits. A second polyclonal antibody was prepared using recombinant bovine leptin prepared as described above. The recombinant protein was gel-purified and the appropriate band excised and used for antibody production.